Original Article

Association of SARS-CoV-2 Viral Load with Biochemical Profile of COVID-19 Patients: A Nigerian Experience

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Abstract

Background: Kidney involvement in coronavirus disease 2019 (COVID-19) pathology has been supported by high frequency of angiotensin-converting enzyme 2 (ACE2) expression on renal cells and reports of acute kidney injury. However, the association between host viral load and kidney function is not clear. **Aim:** In this study, plasma levels of renal markers (urea nitrogen, creatinine, and estimated glomerular filtration rate (eGFR)) and electrolytes (sodium, potassium, chlorine, and bicarbonate) were assessed in relation to SARS-CoV-2 viral load of COVID-19 patients. **Patients and Methods:** This cross-sectional study involved 144 consenting COVID-19 patients admitted to the Ogun state COVID-19 isolation center between May and December 2020. All participants presented with mild respiratory symptoms and did not require ICU admission or ventilation support. Data included reverse transcriptase polymerase chain reaction (RT-PCR) cycle threshold (C_T) value, blood urea nitrogen (BUN), creatinine, sodium, potassium, chlorine, bicarbonate measurements, and glomerular filtration rate. Reference intervals were used as comparators, and multiple linear regression model was fitted. Statistical significance was set at $P < 0.05$. **Results:** BUN level and creatinine were elevated in 4 (2.8%) and 42 (29.2%) patients, respectively, with lowered eGFR observed in 37 (25.7%) patients. Hyponatremia and hypokalemia were observed in 35 (24.3%) and 21 (14.6%) patients, respectively, while hypochloremia was observed in 21 (14.6%) patients. Lowered bicarbonate was observed in 29 (20.1%) patients. Linear regression showed statistically significant association ($R^2 = 0.340$, $P = 0.032$) between RT-PCR C_T value and eGFR ($\beta = 0.006$, $P = 0.017$) as well as HCO_3 ($\beta = -0.262$, $P = 0.036$). **Conclusion:** COVID-19 patients with mild respiratory symptoms exhibited renal abnormalities, electrolytes, and acid-base imbalances which were partly associated with SARS-CoV-2 viral load.

Keywords: *COVID-19, electrolytes, Nigeria, renal markers, viral load*

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Introduction

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily affects the human respiratory system but has also exhibited multisystem involvements in its etiology and progression.[1] The virus is transmitted through respiratory droplets, contact, and fomites,[2]

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and it gains access to the host cells by binding to membrane-bound angiotensin-converting enzyme 2 (ACE2).[3] Although ACE2 is highly expressed in the lower lungs on type I and II alveolar epithelial cells,[4] there is evolving evidence of its expression in other organs, including the kidney, heart, and gut.[5,6] Involvement of these organs in COVID-19 prognosis has been supported by reports that acute kidney injury (AKI), cardiac damage, and abdominal pain are commonly reported co-morbidities of COVID-19.[7] This suggests that SARS-CoV-2 may have a tropism for non-alveolar (extra-pulmonary) ACE2-expressing cells such as cells of the kidney.

High frequency of ACE2 expression in the brush border of proximal tubular cells as well as podocytes of the kidney, though to a lesser extent, has been previously demonstrated.[5] Diao and colleagues[8] examined viral nucleocapsid protein *in situ* in the kidney post-mortem and found that SARS-CoV-2 antigens accumulated in kidney tubules. Xu *et al*.[9] also suggested that SARS-CoV-2 infects the human kidney, inducing AKI, and contributes to viral spread in the body.[9] Although these are indicative of involvement of renal cells in COVID-19 pathology. However, the reported incidence of acute renal injury was 3%–7% from an estimated 33% incidence of organ dysfunction in COVID-19.[10,11] Furthermore, reports of AKI incidence among COVID-19 patients have been largely inconsistent, ranging from 0.1%–29%,[10-13] with the factors that predispose COVID-19 patients to renal injury yet to be identified. Though a previous study[14] posited that renal disorders in coronavirus infection could be due to dehydration, sepsis leading to cytokine storm syndrome, rhabdomyosis and hypoxia, it is not clear if these are applicable to the recent SARS-CoV-2 infection since the study was on SARS-CoV infection.

Renal disorders are associated with impaired renal function, which could lead to obstruction of metabolites and toxins excretion. This adversely affects the maintenance of body electrolyte concentration and acid-base balance.[15,16] These imbalances could have important implications for patient management in regard to identification of potential pathophysiologic mechanisms, and they could also drive novel diagnostic, therapeutic, and prognostic opportunities. Hypokalemia was reported in COVID-19 patients in China,[10] while hyponatremia was reported in COVID-19 patients in the United States.[16] It is not clear if these are secondary to COVID-19-associated renal disorders or a direct effect of SARS-CoV-2 viral load.

The cycle threshold (C_T) value derived from reverse transcriptase polymerase chain reaction (RT-PCR) test provides an estimate of SARS-CoV-2 viral load and is currently widely accepted and utilized for COVID-19 diagnosis.[17] The value indicates the number of thermal cycles at which the fluorescent signal exceeds background signal and thus passes the threshold for positivity. Typically, RT-PCR assays have a maximum of 40 thermal cycles. Samples with higher quantities of viral genetic materials tend to produce fluorescent signal exceeding background signal at early thermal cycles thereby indicating an inverse association between RT-PCR C_T value and viral load. A C_T value of less than 40 is defined as a positive test, while a C_T value of 40 or more is defined as a negative test.[17] Some studies have demonstrated associations between SARS-CoV-2 RT-PCR C_T value and mortality, disease progression, transmission potential, pneumonia severity, hypoxemia intensity, and risk of death.[18-22]

This present study assessed plasma levels of renal markers (urea nitrogen, creatinine, and estimated glomerular filtration rate (eGFR)) and electrolytes (sodium, potassium, chloride, and bicarbonate) in relation to SARS-CoV-2 viral load of COVID-19 patients before treatment commencement.

Methods

Participants

A total of 144 consenting RT-PCR-confirmed COVID-19 patients (SARS-CoV-2 positive) were recruited for this cross-sectional study following case confirmation and admission to the Ogun state COVID-19 isolation center. Study participants comprised 93 males (64.6%) and 51 females (35.4%) within the age group of 12–95 years (mean age 37.32 ± 15.39). They presented with mild respiratory symptoms and did not require ICU admission or ventilation support. Individuals with previously diagnosed renal dysfunction, hypertension, diabetes, human immunodeficiency virus infection, and pregnant women were excluded from the study.

Study location

Patients were recruited from the Ogun state COVID-19 Isolation Centers in Abeokuta, Sagamu, and Ikenne Remo while sample processing and analysis were carried out at the Department of Chemical Pathology and Immunology, Federal Medical Centre (FMC), Abeokuta, Ogun state, Nigeria between May and December 2020.

Ethical consideration

The study protocol was reviewed and approved by the FMC Abeokuta Ethics Committee (FMCA/470/HREC/01/2020/15). A written informed consent was obtained from each participant after careful explanation of the concept or purpose of the study.

Sample collection and storage

Nasal and oropharyngeal swabs were obtained for SARS-CoV-2 testing using synthetic fiber swabs. Nasal sample was obtained by gently inserting the same swab consecutively into each nostril, parallel to the palate. The swab was held in place for 10 seconds to absorb secretion and gently removed. To obtain oropharyngeal sample, patients were instructed to open their mouths wide and tongue depressed with a wooden tongue depressor. The swab was gently inserted through the mouth, avoiding the tongue, and sample was obtained from posterior pharynx. Nasal and orophayngeal swabs were placed into a single appropriately labeled sterile tube containing viral transport media (VTM) immediately after collection. VTM tube was wrapped in adsorbent paper and placed in an appropriately labeled falcon tube, which was transferred into a zip-lock bag and transported to the laboratory in gio-styles surrounded with hard frozen gel packs. Five milliliters (5 mL) of blood sample was aseptically obtained from study participants by venipuncture and dispensed into plain bottles. A trained phlebotomist carried out blood sample collection. Samples were transported in sealed bags placed in an airtight container to the laboratory for processing. Serum was separated by centrifugation at 4000 radians per min, for 10 min in a biosafety cabinet and stored at -20°C till analysis. Ethical standards were carefully observed in the handling, storage, and disposal of research samples.

Laboratory Method

SARS-CoV 2 RT-PCR

RNA extraction

Viral RNA was extracted using Qiagen Viral RNA Extraction kit (Qiagen Inc, Valencia, CA, USA) according to the manufacturer's instruction. 140 µL of VTM containing patient's nasal and oropharyngeal samples was used for RNA extraction and eluted in 60 µL of viral lysis buffer (AVE buffer) supplied in the extraction kit. All procedures were carried out in a class 2 biosafety cabinet and in line with the World Health Organization's general procedures for inactivation of viral pathogens in a BSL3 facility.[23,24]

Quantitative polymerase chain reaction (qPCR) virus detection

SARS-CoV-2 RT-PCR kit (SuperScript™ III Platinum™ One-Step qRT-PCR Kit; ThermoFisher Scientific, USA) with oligonucleotides targeting the envelope (E) gene and RNA-dependent RNA polymerase (RdRp) gene (TIB MOLBIOL LightMix® Modular SARS-CoV, Berlin) were used for this assay. A reaction mix containing: 0.8 mM $MgSO_4$, 0.04 mg/mL BSA, 0.4 µM each of forward and reverse primers, 0.2 µM probe, 1 µL of SSIII/Taq enzyme mix, and 5 µL purified RNA was prepared as indicated. For the RdRp assay, the forward primer concentration was increased to 0.6 µM. Assay was run on Mic qPCR Cycler (Biomolecular Systems, Australia) as follows: 50°C for 20 min, 95°C for 10 min, then 40 cycles of 95°C for 15 seconds and 60°C for 30 seconds. The number of PCR thermal cycles taken to produce a fluorescent signal that exceeds the background signal (C_T value) was recorded.

Renal function markers

Urea nitrogen

Urea nitrogen was measured using the urease and GLDH method on the ARCHITECT C4000 clinical chemistry analyser (Abbott Diagnostics, Abbott Park, IL, USA). The method is an adaptation of the enzymatic method of Talke and Schubert.[25] In this method, urea is hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate are converted to glutamate in a reaction catalyzed by L-GLDH. Simultaneously, a molar equivalent of reduced nicotinamide adenine dinucleotide is oxidized. The rate of change in absorbance at 340 nm, due to the disappearance of NADH, is proportional to the concentration of urea nitrogen in the sample.

Creatinine

Creatinine concentration was measured using the Jaffe rate-blanked creatinine assay on the ARCHITECT C4000 clinical chemistry analyser (Abbott Diagnostics, Abbott Park, IL, USA). This procedure is adapted from the method of Hawk *et al*.[26] Creatinine reacts with picrate ion formed in alkaline medium to develop a red-orange color. The absorbance at 505 nm is compared with that of creatinine standard under the same condition.

Glomerular filtration rate

eGFRs were obtained using the eGFR calculator based on Modification of Diet in Renal Disease study equation[27] as recommended by USA National Kidney Foundation.

Electrolytes

Sodium, potassium, bicarbonate, and chloride (Na, K, HCO3, Cl)

Serum concentrations of Na, K, and Cl were measured using the ion selective electrode (ISE) method on the SFRI ISE 6000 electrolyte analyser (SFRI Medical diagnostics, Saint Jean d'Illac, France). This method uses the unique properties of membrane materials to develop an electrical potential for the measurement of ions in solution.

Statistical analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 (International Business Machines Corporation, Armonk, NY, USA). Categorical variables were summarized as frequency and percentage while continuous variables were summarized as mean and standard deviation. Multiple linear regression model was fitted to estimate the association between SARS-CoV-2 viral load by RT-PCR C_T values with biochemical parameters of patients. Patients were divided into two groups based on their RT-PCR C_T values, and the mean of biochemical parameters were compared using Student t-test and Mann–Whitney U test for parametric and non-parametric variables, respectively. A two-sided α of less than 0.05 was considered statistically significant.

Results

Study participants comprised 93 males (64.6%) and 51 females (35.4%) within the age group of 12–95 years. The mean age of study participants was 37.32 ± 15.39 years and they included 4 (2.8%) teenagers (age 10–17 years), 77 (53.5%) young adults (age 18–35 years), 43 (29.9%) middle-aged adults (age 36–55 years) and 20 (13.9%) older adults (ages >55 years). The mean RT-PCR C_T value of study participants was 30.17 ± 4.96 with a range of 11.58–39.50. The C_T values <30 were observed in 65 (45.1%) study participants while 79 (54.9%) had C_T values between >30. These are described in Table 1.

Table 1: Demographic characteristics and RT-PCR cycle threshold of study participants

Variable	Frequency (*n*)	Percentage
Gender		
Male	93	64.6
Female	51	35.4
Age (years)	37.32±15.39 (12-95)	
Children (1-9 years)	0	0.0
Teenagers (10-17 years)	4	2.8
Young adults (18-35 years)	77	53.5
Middle aged adults (36-55 years)	43	29.9
Older adults (>55 years)	20	13.9
C	30.17±4.96 (11.58-39.50)	
<30	65	45.1
>30	79	54.9

Blood urea nitrogen (BUN) level was elevated in 4 (2.8%) patients. Elevated plasma creatinine levels were observed in 42 (29.2%) patients whereas lowered plasma creatinine levels and eGFR were observed in 8 (5.6%) patients and 37 (25.7%) patients, respectively. Hyponatremia and hypokalemia were observed in 35 (24.3%) patients and 21 (14.6%) patients, respectively while hypernatremia and hyperkalemia were observed in 5 (3.5%) patients, respectively. Hypochloremia was observed in 21 (14.6%) patients while hyperchloremia was observed in 9 (6.3%) patients. Lowered plasma level of bicarbonate was observed in 29 (20.1%) patients. These are depicted in Table 2.

Linear regression with RT-PCR C_T value as dependent variable and renal function markers and electrolytes as predictors showed statistically significant association (R^2 = 0.340, P = 0.032) between C_T value and eGFR (β = 0.006, P = 0.017) as well as HCO_3 (β = -0.262, P = 0.036). Thirty-four percent (34%) of the observed data fitted into the linear regression model obtained (R^2 = 0.340, P = 0.032). This is shown in Table 3. Patients were stratified based on their C_T value into two groups, C_T <30 and C_T ≥30. Mean comparison of variables between the groups showed statistically significant differences in age, eGFR, and HCO_3. Mean comparison of variables between the groups showed statistically significant differences in eGFR and HCO3. Mean eGFR was significantly lower in the group CT <30 compared with CT ≥30 whereas mean HCO3 was significantly higher in the group CT <30 compared with CT ≥30. This is shown in Table 4.

Discussion

Renal abnormalities including proteinuria, hematuria, and AKI have been associated with moderate to severe COVID-19 cases.[28] There is also growing evidence that SARS-CoV-2 can infect podocytes and tubular epithelial cells of the kidney.[8] However, data on kidney function impairment have been largely inconsistent in

Table 2: Renal function markers and electrolytes levels of study participants

Variable	Reference interval (RI)	Within RI	<LRL	>URL	Total (<LRL + >URL)
BUN (mg/dL)	8-20	140 (97.2)	0 (0.0)	4 (2.8)	4 (2.8)
Creatinine (mg/dL)	0.6-1.2	94 (65.3)	8 (5.6)	42 (29.2)	50 (34.7)
eGFR (mL/min/1.73 m^2)	≥90	107 (74.3)	37 (25.7)	-	37 (25.7)
Na (mmol/L)	135-145	104 (72.2)	35 (24.3)	5 (3.5)	40 (27.8)
K (mmol/L)	3.5-5.1	118 (81.9)	21 (14.6)	5 (3.5)	26 (18.1)
Cl (mmol/L)	96-106	114 (79.2)	21 (14.6)	9 (6.3)	30 (20.8)
HCO_3 (mmol/L)	20-30	115 (79.9)	29 (20.1)	0 (0.0)	29 (20.1)

the context of mild COVID-19 cases. In the present study, COVID-19 patients studied presented with mild respiratory symptoms and did not require ICU admission or ventilation support at presentation. Nonetheless, elevated BUN and serum creatinine were observed in 2.8% and 29.2% of these patients, respectively, with reduced eGFR observed in 25.7% of the patients. These suggest that kidney abnormalities in mild COVID-19 cases are plausible, and that the manifestation of these abnormalities in mild disease may be subclinical, hence the inconsistency in reports.

Serum levels of creatinine and urea reflect the filtration capacity of the glomerulus, which is an indication of kidney function.[29] Elevated creatinine level, above reference interval, is almost invariably a consequence of reduced glomerular filtration rate with the implication of an inherent renal cause. However, urea concentration elevation above reference interval has also been associated with decreased GFR, but other non-renal conditions could also result in elevated urea concentration.[29] Our finding of elevated BUN in 2.8% of COVID-19 patients agrees with the report of Xiang *et al*.[30] who observed elevated BUN in 2.6% of COVID-19 patients at admission. Xiang *et al*.[30] also reported elevated serum creatinine and decreased eGFR in 0.6% and 3.9% of patients, respectively, which is less than our finding of elevated serum creatinine and decreased eGFR in 29.2% and 25.7% of the patients, respectively. The higher prevalence of renal involvement in COVID-19 disease in our study population could be due to genetic differences between individuals in our study and previously reported studies.

Izzedine *et al*.[31] reported collapsing glomerulopathy with acute tubular necrosis during SARS-CoV-2 infection in patients of African ancestry homozygous for the *APOL1* G1 allele variant. They posited that SARS-CoV-2 plays a role in the development of collapsing glomerulopathy in individuals with this genetic risk factor and proposed the term COVID-19-associated nephropathy. Indeed, Nigerians have a relatively higher risk of SARS-CoV-2-associated nephropathy, given the previous reports that the highest APOL1 G1 allele frequencies are found in Ghana and Nigeria.[32] However, there is a need for further studies to determine this association. Other mechanisms proposed to explain SARS-CoV-2-associated renal injuries include renal hemodynamic modifications, endocrine dysregulation as well as massive release of inflammatory cytokines which result in tubular and glomerular cell injuries.[33]

SARS-CoV-2 gains entry into cells through ACE2, a key enzyme in the renin-angiotensin system (RAS), which plays a significant role in regulating fluid and electrolyte balance.[34] In combination with renal dysfunction and volume changes resulting from gastrointestinal fluid loss due to diarrhea and vomiting,[34] COVID-19 patients are at increased risk of fluid and electrolyte imbalances. In this present study, hypokalemia, hyponatremia, and hypochloremia were observed in 24.3%, 14.6%, and 14.6% of the patients, respectively. This agrees with previous reports of fluid and electrolyte imbalances in COVID-19 patients[16,28,35,36] and could be due to renal dysfunction,[36] disruption of the RAS leading to increased angiotensin II concentration,[35,37] increased release of antidiuretic hormone in response to volume depletion following gastrointestinal fluid losses.[28] This present study also found decreased serum bicarbonate concentration in 20.1% of COVID-19 patients. Our

Table 3: Multiple linear regression model of RT-PCR C_T value with renal function markers and electrolytes levels of study participants

Model summary	R^2=0.340	F=2.190	P=0.032*
Variable	Beta	SE	P
BUN (mg/dL)	−0.052	0.053	0.325
Creatinine (mg/dL)	1.334	0.763	0.083
eGFR (mL/min/1.73 m^2)	0.006	0.002	0.017*
Na (mmol/L)	−0.007	0.074	0.922
K (mmol/L)	1.186	0.793	0.137
Cl (mmol/L)	0.013	0.108	0.905
HCO_3 (mmol/L)	−0.262	0.124	0.036*

*Significant at P<0.05

Table 4: Mean comparison of renal function markers and electrolytes levels between patients with CT <30 and CT ≥30

Variable	C_T <30 (n=65)	C_T ≥30 (n=79)	t	P
BUN (mg/dL)	12.48±3.89	12.55±3.82	−0.101	0.920
Creatinine (mg/dL)	0.87±0.37	0.84±0.87	−0.365#	0.715
eGFR (mL/min/1.73 m^2)	167.08±159.29	271.20±236.77	−2.955#	0.003*
Na (mmol/L)	132.58±4.95	133.01±8.43	−0.364	0.717
K (mmol/L)	3.97±0.60	4.02±0.61	−0.506	0.614
Cl (mmol/L)	99.74±4.79	99.83±5.35	−0.103	0.918
HCO_3 (mmol/L)	23.45±3.38	21.37±3.66	3.509	0.001*

*Significant at P<0.05. #Z score: Mann-Whitney U non-parametric test

finding is in line with previous reports of acid-base disturbance in the form of metabolic acidosis in COVID-19 patients.[37-39] It is postulated that metabolic acidosis in COVID-19 patients is caused by hypercapnia and organ hypoperfusion.[40]

Multiple linear regression analysis of SARS-CoV-2 viral loads estimated by the C_T value, with BUN, creatinine, eGFR, and electrolytes as predictors showed significant associations between C_T value and eGFR as well as bicarbonate. The linear regression model obtained rightly predicted 34% of the observed data in this present study. There was a direct association between C_T value and eGFR, which indicates that as C_T values decreased (higher viral loads), the eGFR of COVID-19 patients also decreased in this study. This was also supported by significantly lower mean eGFR in patients with C_T value <30 compared to patients with C_T value ≥30. Though this may suggest increased tendency toward renal involvement culminating in reduced eGFR in individuals presenting with high viral loads at diagnosis, further studies are needed to clearly understand the nature of the link between SARS-CoV-2 viral load and changes in glomerular filtration rate. This present study also found an inverse association between C_T value and serum bicarbonate concentration. By implication, patients with lower C_T values (higher viral load) presented with higher bicarbonate levels. This may be a compensatory response to shore up bicarbonate levels in the presence of COVID-19-related metabolic acidosis. However, it is possible that this compensatory response requires a certain threshold, which is not achieved in patients with lower SARS-CoV-2 viral load. Further investigations are required to validate these postulates.

Conclusion

The present study provides evidence of renal abnormalities, electrolytes, and acid-base imbalances in COVID-19 patients presenting with mild respiratory symptoms at diagnosis, as well as significant associations between SARS-CoV-2 viral load, estimated glomerular filtration rate, and serum bicarbonate levels.

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Conflicts of interest

There are no conflicts of interest.

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